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Mechanisms of the Biological Effects of PCBs, Polychlorinated Dibenzo-*p*-dioxins and Polychlorinated Dibenzofurans in Experimental Animals

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Polychlorinated biphenyls, certain polychlorinated dibenzo-*p*-dioxins and certain polychlorinated dibenzofurans cause a variety of biological effects in experimental animals. The mechanism of the induction of certain enzymes is perhaps best understood. That is, there is binding of certain chlorinated biphenyls, dibenzo-*p*-dioxins and dibenzofurans to a receptor, translocation of the compound–receptor complex into the nucleus followed by an increased activity of a number of enzymes in the cell.

Although the concentration of this receptor in various tissues of some mouse strains correlates well with the intensity of some of the biological effects observed in the mouse strains exposed to these compounds, this correlation apparently does not extend across various species. The current evidence suggests that the acute toxic effects of TCDD in various species is in some way associated with binding of TCDD to the receptor. However, biological effects of TCDD in addition to those resulting from binding to the receptor may be required to produce acute toxicity and, perhaps, other effects.

The acute toxic effects of TCDD are probably caused by the parent compound rather than metabolites; however, this conclusion must be viewed as tentative. Also, it cannot be excluded at this time that biological effects other than acute toxicity may be caused by metabolites of TCDD. Finally, the acute toxic effects of TCDD appear not to be related, at least not directly, to the rate of metabolism of TCDD in experimental animals nor to the half-life of excretion.

Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) have enjoyed widespread use in commerce. The most important uses are as plasticizers, transformer fluids, hydraulic fluids and flame retardants. Polychlorinated dibenzofurans occasionally occur as contaminants of samples of PCBs. The chlorinated dibenzo-*p*-dioxins are formed as by-products in the synthesis of polychlorinated phenols. The polyhalogenated dibenzofurans and dibenzo-*p*-dioxins also occur as products of combustion (*1,2*).

Commercial PCBs and PBBs produce a wide variety of biological effects in experimental animals. The most important of these include enzyme induction and inhibition (*3*), decreased reproductive efficiency (*4*), changes in liver morphology (*5*), changes in plasma lipid concentrations (*6*), hepatic porphyria (*7*) decreased immunocompetence (*8*), dermatological effects (*9*) and production of tumors in the livers of rodents (*10*).

Although a great deal is known about the dose-response relationship of commercial PCBs regarding their various biological effects in experimental animals, the biological mechanism of these effects is less well understood. It is apparent that a portion of the induction of certain enzymes by a few of the individual isomers of the PCBs is because of the affinity of these isomers for a receptor protein in the cytosol of mammalian cells which controls the activity of enzymes associated with the regulator gene referred to as the *Ah* locus (*11*). However, the majority of the PCB isomers present in commercial mixtures have no affinity for this receptor protein although they have the capacity to increase the activity of certain enzymes in mammalian cells. Thus, the biological mechanism of the majority of the enzyme induction produced by commercial PCBs is not understood at present.

There is evidence that PCBs may be causing increases in rodent liver tumors largely by mechanisms which do not involve permanent changes in the phenotypic expression of hepatocytes (*12–14*). In other words, they may be acting as promoters.

The mechanism by which PCBs cause hepatic porphyria is the best understood of all the biological effects of this mixture of compounds. The biological event leading to the hepatic porphyria appears to be the inhibition of uroporphyrinogen decarboxylase, the enzyme that is

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responsible for the stepwise decarboxylation of uroporphyrinogen to coproporphyrinogen (*15*). The inhibition of this enzyme leads to the increased accumulation and excretion of porphyrins containing a number of carboxyl groups. Interestingly, Elder and Sheppard (*16*) have recently shown that the decrease in the catalytic activity of the uroporphyrinogen decarboxylase activity seen with the porphyrogenic agent hexachlorobenzene is accomplished without a decrease in the amount of immunoreactive enzyme protein.

Certain isomers of the polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans produce a number of biological effects in experimental animals. The most prominent of these are enzyme induction, lethality, a wasting syndrome, lymphoid involution, hepatic damage (in some but not all species), chloracne (in a limited number of species), hepatic porphyria, gastric lesions and urinary tract hyperplasia (again only in some species), edema (in certain select species), hyperlipidemia, reproductive toxicity, teratogenic effects and the induction of an increase in tumor incidences in various organs of rats and mice (*17*). The concentrations of the select chlorinated dibenzo-*p*-dioxins and dibenzofurans that cause these biological effects are almost always many orders of magnitude lower than the concentrations of commercial PCBs required to cause the same or similar biological effects.

The remainder of the discussion will be largely confined to the mechanisms of toxicity of the polychlorinated dibenzo-*p*-dioxins and more specifically to the specific isomer 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). There are 75 possible isomers of the chlorinated dibenzo-*p*-dioxins. The isomer most biologically active in experimental animals appears to be TCDD.

The single dose of TCDD which produces acute lethality in a number of animal species varies quite widely (Table 1). Of the animal species so far examined, the guinea pig (2 μg/kg) (*18*) and the hamster (>3000 μg/kg) (*19,20*) occupy the extremes. The LD_{50} for TCDD to the remainder of the animals that have been examined is between these two extremes. The LD_{50} of TCDD varies by sex in the Sprague-Dawley rat, with the female (25 μg/kg) being more susceptible than the male (60 μg/kg) (*21*). The LD_{50} also varies with age in the rat, with the weanling male (25 μg/kg) being more susceptible than the adult male (60 μg/kg) (*21*). There is a variation in LD_{50} by strain in mice with the C57BL/6J mouse (132 μg/kg) being more susceptible than the DBA/2J (620 μg/kg) (*22*). As noted in Table 1, the LD_{50} for rabbits also varies by route of administration (*23*).

Table 1. Single dose LD_{50} values for TCDD.

Species	Route	LD_{50}, μg/kg	Reference
Guinea pig	Oral	2	(*18*)
Monkey	Oral	50	(*18*)
Rat			
Adult male	IP	60	(*21*)
Weanling male	IP	25	(*21*)
3-MC pretreated weanling male	IP	44	(*21*)
Adult female	IP	25	(*21*)
Rabbit	Oral	115	(*23*)
Rabbit	Skin	275	(*23*)
Mouse			
C57BL/6J	IP	132	(*22*)
DBA/2J	IP	620	(*22*)
B6D2F1/J	IP	300	(*22*)
Hamster	IP	> 3000	(*19*)
Hamster	Oral	5051	(*20*)

Table 2. Enzymes induced by TCDD in experimental animals.

Cytochrome P-450
UDP-glucuronyltransferase
DT-diaphorase
Ornithine decarboxylase
α-Aminolevulinic acid synthetase
Glutathione-S-transferase B
τ-Aldehyde dehydrogenase
Choline kinase

Of the various biological effects of the chlorinated dibenzo-*p*-dioxins and dibenzofurans, the mechanism of induction of the activity of enzymes is best understood. Listed in Table 2 are some of these enzymes whose activities are temporarily increased in animals exposed to TCDD and certain other chlorinated dibenzo-*p*-dioxins, dibenzofurans and PCB isomers (*17*). The increase in the activity of at least some of these enzymes seen on administration of TCDD and certain related compounds to experimental animals or incubation of TCDD with certain cells in culture, apparently results from a binding of TCDD to a receptor protein in the cell, and the translocation of this TCDD-receptor complex into the nucleus (*17*). In the nucleus this TCDD-receptor complex apparently binds to a regulatory gene which controls the concentration of most if not all of these enzymes in the cell. This regulatory gene is often referred to as the *Ah* locus after Nebert (*24*). Although a number of workers have contributed to our understanding of this process, the original observations have come from the laboratory of Alan Poland and his colleagues (*25*).

Poland has carried out a number of studies correlating the binding of various chlorinated dibenzo-*p*-dioxins to this receptor protein with the biological activity of these same compounds (*26*). One such study was a comparison of the relative affinity of TCDD and other chlorinated dibenzo-*p*-dioxins of TCDD for the receptor protein in the liver cytosol of C57 black 6 mouse with the ability of these compounds to induce the activity of the P-450 enzyme, AHH, in chick embryos. These experiments showed a good correlation between affinity of the compounds for the receptor and their ability to induce AHH in the chick liver.

This study also demonstrated that only those chlorinated dibenzo-*p*-dioxins in which at least three of the four lateral positions (2,3,7 or 8) on the dibenzo-*p*-dioxin ring system are occupied with chlorine atoms have an appreciable ability to both bind to the receptor and to induce AHH in chick embryos at the concentrations

used in these studies. The acute lethality of these chlorinated dibenzo-*p*-dioxin isomers in a specific species of experimental animal (*18*) generally follows the same structure–activity relationship shown in this study for receptor binding and induction of chick embryo AHH (*17*). Additional work in a number of laboratories using different strains of mice in which there are variable levels of the receptor, as detected by incubation of liver cytosol with ^{3}H-TCDD, have found that the level of the receptor or the affinity of the receptor for TCDD also correlates with the ability of TCDD to induce the activity of AHH (*17*) to cause acute toxicity (*27*), to bring about thymic involution (*28*), to produce cleft palate (*28*) or induce hepatic porphyria (*29*). However, this correlation between the level of the receptor and biological effects seen in the mouse strains does not hold when various species are compared.

A comparison of the concentrations of the TCDD receptor in the rat and various strains of mice has recently been published (*30*) (Table 3). With the exception of the concentrations of the receptors in the livers of the DBA mouse and the cross between the C57BL/6J and the DBA mice, the B6D2F1/J mouse, there are little or no differences between the mouse strains examined and the Sprague-Dawley rat.

This comparison has been recently expanded (*31*). This more recent study has shown that the level of the receptor in the liver of the guinea pig is not significantly different than that in the Sprague-Dawley rat, the cynomolgus (*Macaca fascicularis*) monkey, the C57BL/6J mouse or the Syrian golden hamster. In addition, the affinity of TCDD for the receptor in these species did not appear to be significantly different. TCDD does not increase the activity of the *Ah* locus enzymes, AHH and DT-diaphorase in the guinea pig (*32*), the most sensitive species to the acute toxicity of TCDD. Thus, in contrast to the data in various mouse strains, there does not appear to be a correlation between enzyme induction and the presence of the TCDD-receptor in the guinea pig. Also, although the concentrations of receptors in the liver and the affinity of TCDD for these receptors are very similar in the various species, the acute toxicities are quite different. Recall, for example, that the LD_{50} of TCDD in the guinea pig is 2 μg/kg whereas in the hamster it is >3000 μg/kg (Table 1). A possible reason for the reduced acute lethality of TCDD in the hamster or its inability to induce the enzymes of the *Ah* locus in guinea pigs may be that the TCDD-receptor complex may not be transferred from the cytosol to the nucleus in these two species. However, data from the laboratory of Gasiewicz (personal communication) indicate that under the same *in vivo* conditions, TCDD is translocated into the nucleus of the rat, mouse, hamster and the guinea pig in similar amounts. Thus, there are inconsistencies across species in the concept that the affinity of TCDD and, perhaps, other chlorinated dibenzo-*p*-dioxins and dibenzofurans for the cytosol receptor as well as the concentration of the receptor is related to the ability of these compounds to induce various enzymes and to cause other toxic effects including liver damage and acute lethality. In spite of these inconsistencies, the data using various mouse strains (*17,27–29*) and the results of structure activity studies (*26*) suggest that the binding of TCDD and related compounds to the receptor is in some way related to some of the biological effects of TCDD seen in experimental animals. There is no reason to believe that the alteration in the activity of enzymes noted in Table 2 is responsible for the toxic effects which are seen. A number of compounds can induce the activity of these same enzymes but not show the toxic effects seen on exposure to, for example, TCDD. One possible explanation, among others, for these data is that in addition to the binding to the receptor, TCDD causes additional biological effects which are necessary for TCDD to interfere with the normal functioning of the cell, effects for which there are individual species sensitivities. Studies with mammalian cells in culture (*33–36*) have shown that TCDD apparently has little or no effect on mammalian cell division or viability. These data also suggest that the toxicity of TCDD in whole animals may be related to the alteration of two or more biological parameters, one of which may be external to the cells being affected.

Thymic involution is a consistent effect of TCDD in all animals so far examined. Shown in Table 4 are estimations of the concentrations of TCDD required to reduce the thymus weight by 50% in various species (*31*). Note that the doses required to produce thymic atrophy in the guinea pig are much less than the other species examined. However, the levels of the receptor in the thymus does not correlate with these ED values (*31*). Thus, the levels of receptor are higher in the rat thymus than in the guinea pig. Yet a lower dose of TCDD is required to cause thymic involution in the guinea pig. A further comparison of receptor concentrations in various tissues of the guinea pig, the most

Table 3. Concentration (n) and dissociation constants (K_D) of the TCDD receptor in rat and mouse hepatic cytosol.[a]

Species	n, fmole/mg protein	K_D, nM
Sprague-Dawley rat (7)	61 ± 5	0.12 ± 0.03
C57BL/6J mice		
May–July (4)	74 ± 10	0.29 ± 0.01
February–April (3)	47 ± 8	0.29 ± 0.03
DBA/2J mice (3)	ND	ND
B6D2F1/J mice		
February–April (3)	23 ± 2	0.42 ± 0.03

[a]Data of Gasiewicz and Neal (*30*).

Table 4. Ability of TCDD to produce thymic atrophy in different species.[a]

Species	ED_{50}, μg/kg
Guinea pig	0.5–1.0
Rat	15
Mouse (C57BL/6J)	60
Hamster	>300

[a]Data of Gasiewicz (personal communication).

sensitive species to acute toxicity, and the hamster, the most resistant, shows that with the exception of the heart and testes, the concentrations are quite similar (*31*).

Another question of interest is whether TCDD is metabolized and, if so, what is the effect of metabolism on the acute toxicity of TCDD. In other words, what compound is responsible for acute toxicity—the parent compound or a metabolite or metabolites?

Until recently, there was some question whether TCDD was metabolized in animals, particularly since it appears to be a poor substrate for soil bacteria. However, the work of Rose et al. (*37*), Poiger and Schlatter (*38*) and Olson et al. (*39*) have provided convincing evidence that TCDD is slowly metabolized in a number of species.

When 500 μg/kg ^{3}H-TCDD is administered to hamsters and the urine collected for 24 hr starting on day 7 following administration of TCDD, no parent compound was found in the urine during this period (*39*). However, a number of compounds more polar than TCDD were found in the urine, some of which are apparently present as glucuronides (*40*). Also, some of the metabolites observed were likely ethereal sulfate derivatives of TCDD. An examination of the bile collected from these same animals again reveals no parent compound but a number of metabolites of TCDD, some of which appear to be glucuronide derivatives.

Incubation of ^{3}H-TCDD with primary hepatocytes isolated from hamsters and rats leads to the accumulation of a number of metabolites of TCDD in the incubation media, some of which appear to be glucuronides (*40,41*). Additional data indicate the presence of ethereal sulfate derivatives of TCDD. Similar results to these have also been obtained using hepatocytes isolated from rats, hamsters and mice (*40*).

The major metabolites formed on incubation of primary rat hepatocytes with TCDD are 1-hydroxy-2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 8-hydroxy-2,3, 7-trichlorodibenzo-*p*-dioxin (*41*). These two metabolites represented about 60% of the metabolic products present in the incubation.

It is logical that the enzyme responsible for the formation of the metabolites of TCDD seen on incubation with primary rat hepatocytes is the cytochrome P-450 monooxygenase system. In order to verify this, the effect of pretreatment of rats with the cytochrome P-450 inducer, phenobarbital (Pb) on the ability of the hepatocytes to metabolize TCDD was examined (*40*). It was found that pretreatment of rats with Pb leads to marked increase in the rate of metabolism of TCDD by primary hepatocytes as compared to controls. Also, pretreatment with a small dose (5 μg/kg) of TCDD also markedly increased the rate of metabolism of TCDD by primary rat hepatocytes. When rat hepatocytes were incubated with TCDD in the presence of SKF 525-A (0.1 mM) or metyrapone (0.5 mM), the metabolism of TCDD to the phenolic derivatives.

In order to assess whether the parent compound or metabolites were responsible for the acute toxicity, the LD_{50} of TCDD in weanling rats and in weanling rats pretreated with Pb (50 mg/kg/3 days), 3-methylcholanthrene (3-MC) (40 mg/kg), and TCDD (5 μg/kg) was determined (Table 5) (*21*). In the rats pretreated with Pb, 3-MC and TCDD, the LD_{50} was increased relative to controls. These data suggest that metabolism leads to a decrease in the acute toxicity. This is reinforced by data from the work of Poiger and Buser (*42*) who administered the metabolites of ^{3}H-TCDD excreted in the bile of a dog to guinea pigs. On a molar basis (based on radioactivity), the metabolites in the bile were >100 times less toxic than TCDD itself.

Table 5. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to male weanling rats pretreated with phenobarbital (Pb), 3-methylcholanthrene (3-MC), or TCDD.[a]

Treatment	LD_{50}, μg/kg, mean ± SE
None	25.2 ± 1.4
Pb	40.9 ± 1.3[b]
3-MC	44.1 ± 1.2[b]
TCDD	36.8 ± 1.8[b]

[a] Data of Beatty et al. (*21*).
[b] Significantly ($p < 0.05$) different from controls (none).

Table 6. Rates of elimination, AHH induction and toxicity of TCDD in various species.[a]

Species	Dose, μg/kg	Half-life for elimination $t_{1/2}$, days	AHH induction	LD_{50}, μg/kg	Reference
Guinea pig	2.0 (IP)	30	No	2	(*43*)
Rat	1.0 (Oral)	31	Yes	60	(*37*)
Mouse					
C57BL/6J	10.0 (IP)	17	Yes	132	(*22*)
DBA/2J	10.0 (IP)	37	Yes	620	(*22*)
B6D2F1/J	10.0 (IP)	17	Yes	300	(*22*)
Hamster	650 (IP)	11	Yes	> 3000	(*19*)
Hamster	650 (Oral)	15	Yes	5051	(*19,20*)

[a] Data of Gasiewicz et al. (*43*).

Shown in Table 6 are the half-lives of elimination of TCDD in various species compared with the LD_{50} of TCDD in those same species (*43*). Note that the half-life for elimination of TCDD in the guinea pig and rat are the same, yet the LD_{50} values are quite different. Note also that in the various mouse strains, the strain with the longest half-life of elimination, the DBA, is the least sensitive to the acute lethal effects. Also, in the hamster, which is quite resistant to the acute lethal effects of TCDD, the half-life of elimination of TCDD is not greatly different than, for example, the C57/B6 mouse. These data indicate the acute lethality of TCDD is apparently not directly related to the residence time of TCDD in the organism. And, since residence time is apparently related, at least in part, to the rate of metabolism of TCDD, the acute toxic effects are probably not directly related to the rate of metabolism of the compound.

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